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Mechanism of action of an antioxidant active packaging prepared with Citrus extract

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ABSTRACT

Active packaging consisting of polyethylene terephthalate (PET) trays coated with a Citrus extract, without and with plasma pre-treatment, can reduce lipid oxidation in cooked meat. The mechanism of action of the packaging was investigated by quantifying the extent of transfer of antioxidant components from the active packaging into cooked turkey meat. Kinetic studies revealed the affinity for water of phenolic compounds and carboxylic acids in the Citrus extract, suggesting their diffusion into the water phase of the meat facilitated their antioxidant effect. Analysis by high-performance liquid chromatography permitted the identification of carboxylic acids and flavanones as major components of the extract. Their quantification in meat after contact with the trays revealed a release of 100% of the total coated amount for citric acid, 30% for salicylic acid, 75% for naringin and 58% for neohesperidin, supporting the release of these components into cooked meat as a mechanism of action of the antioxidant active packaging.

Keywords: Citrus extract; PET tray; carboxylic acid; flavanones; HPLC.

INTRODUCTION

Interest in active packaging as an approach to improve the quality and increase the shelf-life of food products has grown (De Kruijf, Van Beesty, Rijky, Sipiläinen-Malm, Paseiro Losada, & De Meulenaer, 2002). Active packaging may be particularly useful in processed food products, such as meat products, which can rapidly deteriorate due to dehydration, discoloration, bacterial growth or degradation processes like lipid oxidation (Seideman, Cross, Smith, & Durland, 1984; Ruban, 2009). Different solutions have been proposed to minimize deteriorative processes in meat products, which include the use of drip or taint adsorbents, oxygen scavengers and carbon dioxide emitters, often used in conjunction with modified atmosphere packaging (Kerry, O’Grady, & Hogan, 2006). These solutions prevent conditions that can cause colour changes, off flavour development and other deteriorative processes in packaged meat. Other solutions include packaging with added active compounds that come in contact with or are released into the food, where they can carry out their protective action (Lee, 2005). Antioxidant active packaging falls into the latter category.

Due to their negative perception among consumers, synthetic antioxidants are being progressively replaced as ingredients in the formulation of antioxidant active packaging by substances of natural origin, such as tocopherol or mixtures of plant and herbal extracts, (Okabe, Watanabe, Shingu, Kushibiki, Hodate, Ishida et al., 2002; Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007). Packaging containing added antioxidants can be prepared by the addition of the active substances to the packaging material before the formation of the plastic film, with a subsequent release of the antioxidants through either diffusion or film degradation (Pettersen, Mielnik, Eie, Skrede, & Nilsson, 2004; Van Aardt,

Duncan, Marcy, Long, O'Keefe, & Sims, 2007). The amount and rate of release of the antioxidant compounds are fundamental to the extent and duration of the protective effect of the packaging; studies have been undertaken on the production of controlled-release packaging that optimizes the characteristics of the plastic polymer to regulate the release of the active substances (Koontz, Moffitt, Marcy, O'Keefe, Duncan, & Long, 2010; Chen, Lee, Zhu, & Yam, 2012;). Some types of active packaging have been prepared by adding the antioxidant substances only to the layer of packaging in direct contact with the food, facilitating the incorporation of the antioxidant in the packaging yet optimizing its release into the food (Camo, Beltrán, & Roncalés, 2008; Soto-Cantú, Graciano-Verdugo, Peralta, Islas-Rubio, González-Córdova, González-León et al., 2008). Alternative systems have been developed by immobilizing the antioxidant compounds in the plastic polymer; these exert their antioxidant activity by trapping free radicals responsible for the initiation and propagation of the lipid peroxidation process (Nerín, Tovar, & Salafranca, 2008).

As cooked meat is highly susceptible to lipid oxidation (Gray, & Pearson, 1987), an immediate interaction of the active compounds with the food could be advantageous to protect the product. A coating of antioxidants on the surface of the packaging in contact with the food can satisfy this requirement and permit an immediate protection against lipid oxidation processes. An antioxidant active packaging developed in our laboratory by coating polyethylene terephthalate (PET) trays with a Citrus extract has already been shown to reduce lipid oxidation in cooked turkey meat (Contini, Katsikogianni, O'Neill, O'Sullivan, Dowling, & Monahan, 2012). The natural antioxidant used is a mixture of carboxylic acids and flavanones and has already been shown to exhibit antimicrobial activity in meat products (Mexis, Chouliara, & Kontominas, 2012). The aim of the present study was to identify the active components in the extract and investigate the mechanism of action of the active packaging. Our hypothesis was that active components in the Citrus extract coated on PET

trays exert their antioxidant activity by migrating from the packaging surface into cooked meat.

2. MATERIALS AND METHODS

2.1 Meat

Turkey breasts (~1.2 kg) were obtained from IGWT Poultry Service Ltd, County Monaghan, Ireland. For the preparation of cooked meat, fresh turkey breast was wrapped in aluminum foil, cooked for ~2 h to an internal temperature of 73 °C and immediately cooled at 4 °C in an ice bath, as described in Contini et al. (2012).

2.2 Reagents

Citrus extract in powder form containing a mixture of flavanones and carboxylic acids was obtained from Citrox Biosciences, Kimbolton, Cambridgeshire, England. A generic composition of the Citrus extract, as per the manufacturer's specification, was naringin 3.6%, neohesperidin 1.9%, rhoifolin 0.4%, poncirin 0.3%, naringenin 0.2%, hesperidin 0.2%, malic acid 15%, ascorbic acid 15% and citric acid 15%. Chloroform ($\geq 99\%$), gallic acid ($\geq 98\%$), methanol ($\geq 99\%$), phosphoric acid ($\geq 99\%$), sodium carbonate ($\geq 99.5\%$), ascorbic acid ($\geq 99\%$), citric acid ($\geq 99\%$), malic acid ($\geq 99\%$), salicylic acid ($\geq 99\%$), hesperidin ($\geq 95\%$), naringin ($\geq 95\%$), neohesperidin ($\geq 90\%$), poncirin ($\geq 95\%$) and rhoifolin ($\geq 99\%$) were obtained from Sigma-Aldrich Ltd, Dublin, Ireland. Recycled polyethylene terephthalate (PET) trays (100 × 150 × 25 mm) were supplied by Holfeld Plastic, Wicklow, Ireland and low-density polyvinylchloride (PVC) catering film (thickness 7.0 μm ; O_2 transmission 2000 $\text{cm}^3 \text{m}^{-2} \text{d}^{-1} \text{bar}^{-1}$) was supplied by Western Plastic Ltd, Galway, Ireland. Screw-cap plastic

tubes (50 ml) were supplied by Sarstedt Ltd, Wexford, Ireland. Membrane filters (0.2 μm) were supplied by Pall Life Sciences, Cork, Ireland.

2.3 Preparation of the PET trays coated with Citrus extract

PET trays were coated with Citrus extract (PET-CIT) by spraying a methanolic solution of the extract onto the polymer surface through a Teflon nebulizer mounted on a computer numerical control system cncGraf (Boenigh Electronics, Bonn, Germany), following the procedure described in Contini et al. (2012). A further set of trays was prepared by a different procedure that involved a pre-treatment of the PET surface, consisting of plasma activation of the tray surface with an atmospheric pressure plasma jet system (PlasmaTreat GmbH, Steinhagen, Germany). Compressed air was used as reagent gas and the plasma procedure carried out using the conditions described in Contini (2013). After the pre-treatment, Citrus extract was nebulized onto tray surface (PET-PA-CIT) as above.

2.4 Measurement of total phenolic components of Citrus extract in meat

Our hypothesis was that if the antioxidant effect of the active packaging required migration of antioxidants from the tray surface into meat then it should be possible to detect Citrus extract components in the meat stored on Citrus coated surfaces. Since phenolic components with known antioxidant effects (Nijveldt, van Nood, van Hoorn, Boelens, van Norren, & van Leeuwen, 2001) are constituents of Citrus extract, an important first step was to establish if it would be possible to detect these phenolics in meat at a level equivalent to that obtained if all the phenolics on the tray surface migrated into the meat. To do this, Citrus extract dissolved in methanol (13.5 mg ml^{-1}) was added to both raw and cooked turkey muscle at a level of 1.35 mg g^{-1} . This level of addition was calculated from the density of the coating applied to the PET tray area in contact with a $30 \times 30 \times 5$ mm slice (3 g) of turkey

meat (Contini et al., 2012). Citrus extract was also added to distilled water for comparison (1.35 mg g⁻¹).

A second experiment was carried out to quantify the release of phenolic compounds into cooked turkey meat in contact with PET, PET-PA, PET-CIT and PET-PA-CIT trays. The meat was cut into 5 mm thick slices using a meat slicer (Medoc, Logroños, Spain) and subsequently cut into 3 g square (30 × 30 mm) pieces which were placed on the trays and stored at 4 °C for 2 days. Meat pieces were removed immediately (day 0) and after 1 and 2 days of storage for measurement of total phenol (TP) content.

The extraction of phenol components from meat was performed following the procedures described by Jang, Liu, Shin, Lee, Lee, Lee et al. (2008). Meat samples were homogenized in 15 ml of distilled water for 1 min at 8000 rpm using an Ultraturrax T25 (IKA-Labortechnik) and subsequently 9 ml of chloroform were added. The mixture was shaken vigorously and centrifuged for 10 min at 3000 rpm for phase separation. The quantification of TP content in the upper aqueous phase was performed by the Folin-Ciocalteu (FC) procedure described by Harbourne, Marete, Jacquier, & O’Riordan (2009). This involved adding 0.2 ml of aqueous meat extract to 0.5 ml of FC reagent, 1.5 ml of 20% sodium carbonate and 7.8 ml of distilled water. The solution was mixed and left for 2 h for colour development. The absorbance was measured using a Shimadzu UV-1240 spectrophotometer (Kyoto, Japan) at a wavelength of 760 nm. Quantification was done based on a standard curve generated with gallic acid (0.01 - 0.5 mg ml⁻¹) and TP content was expressed as mg gallic acid equivalent (GAE) g⁻¹ of meat.

2.5 Kinetics of release of total phenolic components from Citrus extract-coated trays into water

Square pieces of the PET trays (30×30 mm), corresponding to the surface in contact with the meat slices, were cut from uncoated (PET and PET-PA) and coated (PET-CIT and PET-PA-CIT) trays. Each piece was then placed in a weighing boat with 2.1 ml of water which corresponded to the moisture content of 3g of cooked turkey meat (McCance, & Widdowson, 2002). The samples were then stored at 4 °C for 2 days, to mimic the conditions of meat storage. The weighing boats were covered with PVC catering film to prevent evaporation of the water during storage. The TP content in water was determined immediately (day 0), after 10, 20, and 40 seconds, after 1, 2, 5, 10, 20 and 40 minutes, after 1, 2, 3, 4 and 6 hours and after 1 and 2 days, using the FC procedure described in section 2.4.

2.6 Identification and quantification of the Citrus extract components

The identification of Citrus extract components was performed by high-performance liquid chromatography (HPLC) analysis of a solution of the extract and comparing the retention times of the peaks obtained with those of pure standards of the components declared by the provider of the Citrus extract. The Citrus extract (in powder form) was dissolved in methanol (5 mg ml^{-1}), filtered through a $0.2 \text{ }\mu\text{m}$ membrane filter and analysed by HPLC, following the method described by Harbourne et al. (2009) with modifications to the mobile phase. The analysis was carried out using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent Synergi Hydro-RP 80A analytical column ($250 \text{ mm} \times 4.60 \text{ mm}$, $4 \text{ }\mu\text{m}$ particle size) and a C18 guard column (Phenomenex, Cheshire, UK). The mobile phase was (A) 0.1% phosphoric acid in water and (B) methanol. The separation was carried out at 37 °C at a flow rate of 0.8 ml min^{-1} with the following gradient: 0-2 min, 90% A; 2-16 min, 90% A to 10% A; 16-22 min, 10% A to 90% A. The detector used was a diode array (DAD) at a wavelength of 210 nm. For the

quantification of the main Citrus extract compounds identified, the instrument was calibrated with 6 point calibration curves of their pure standards.

For confirmatory purposes, liquid chromatography-mass spectrometry (LC-MS) analysis was performed using a Waters Acquity HPLC system (Milford, MA, USA), coupled with a triple-quadrupole mass-spectrometer Xevo TQ Waters-Micromass (Manchester, UK). The analysis was carried out using the same column and mobile phase described for the HPLC-DAD analysis. The mass spectrometry analysis was performed in negative electrospray ionization (ESI) in scan acquisition mode with a desolvation temperature of 400 °C and the desolvation gas flow of 800 l h⁻¹.

2.7 Quantification of Citrus extract components in cooked meat stored on Citrus extract-coated trays

Turkey meat pieces (see section 2.4) were placed on PET (control) and PET-CIT trays and removed immediately (day 0) or after 1 or 2 days of storage in a refrigerator at 4 °C. After the exposure time, the Citrus extract components released into the meat were extracted using the method of Folch, Lees, & Sloane-Stanley (1957) with modifications. The meat pieces were transferred to 50 ml tubes and homogenized for 3 min at 8000 rpm with 19 ml of chloroform, using an Ultraturrax T25. The mixture was shaken 3 times to dissolve the fat contained in the tissue, 4 ml of distilled water were then added and the mixture shaken and centrifuged for 10 min at 3000 rpm. An aliquot (2 ml) of the aqueous upper phase was filtered through a 0.2 µm membrane filter and 10 µl were injected onto the HPLC column. The recovery of the extraction was calculated as 61% for naringin, 64% for neohesperidin, 100% for citric acid and 53% for salicylic acid. Organic acids and flavanones were determined as described above (see section 2.6).

2.8 Quantification of Citrus extract components remaining on coated trays after contact with meat

To confirm the extent of release of Citrus extract components into the meat, the components remaining on the trays after exposure to cooked turkey meat slices for up to 2 days at 4 °C (see section 2.4) were quantified. Pieces (30 x 30 mm) of PET-CIT trays, previously in contact with the slices of meat, were cut and placed for 2 hours in weighing boats containing 4 ml of water which corresponded to the final volume of the extract from meat (see section 2.7). Pieces of PET-CIT trays of the same size which had not been in contact with the meat were also immersed in 4 ml of water for 2 hours to act as a control. An aliquot of the water was then collected from each weighing boat, filtered through a 0.2 µm membrane filter and injected onto the HPLC column. The quantification of the components was performed following the procedure described in section 2.4. The amount of the Citrus extract components released into the meat was calculated as the difference between the release into water from PET-CIT trays after contact with the meat and the release from PET-CIT trays that had not been in contact with the meat (control).

2.9 Statistical analysis

Each experiment was carried out in triplicate, values were expressed as mean ± standard deviation of the three repetitions. A one-way analysis of variance (ANOVA) and Bonferroni's pair wise comparison test were used to determine significant differences between the treatments, using SPSS (version 18) statistical software (IBM Inc. Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Direct addition of Citrus extract to raw and cooked meat

In the first experiment, the raw minced meat without the addition of Citrus extract (control) had approximately 1.35 ± 0.07 mg GAE g⁻¹ of meat at day 0 and it did not change significantly at day 1 and day 2 (Figure 1a). The response to the FC reagent in the control meat is most likely due to of its reaction with substances naturally present in the meat. In fact, meat is a complex matrix that contains different classes of substances which can react with the FC reagent. This is supported by previous studies which showed that sugars, aromatic amines, organic acids and Fe (II) can interfere in the reaction (Szydłowska-Czerniak, Tułodziecka, & Szlyk, 2012). Contributions to the FC reaction have also been attributed to amino acids, proteins and inorganic substances (Prior, Wu, & Schaich, 2005). Furthermore, the reaction of the phenolic group of tyrosine with the FC reagent is also well known and the basis of an assay for the quantification of soluble proteins (Lowry, Rosebrough, Lewis Farr, & Randall, 1951); thus the presence of phenolic amino acids, in particular, would have contributed to the relative high FC response of the control. The response in the meat with added Citrus extract showed higher values compared to the control meat on each of the days, however, the difference was significant only at day 2 ($p < 0.05$). The difference between the values found in the control meat and meat with added Citrus extract was lower than the value obtained in distilled water to which the same amount of Citrus extract had been added. If all the phenolics in the added Citrus extract were readily extractable and detectable, then one would have expected the differences between the level of TP in the meat with added Citrus extract and that in the control meat to be approximately equal to the level of TP in the water control. As it was, the difference between Citrus extract added meat and control meat was only approximately 40-50% of the value in water, this lower than expected level might be due to the effect of the components in meat decreasing the efficiency of FC reaction or giving rise to an incomplete recovery of the phenolic components of Citrus extract in the procedure for

their extraction from the meat. The lower variability of data obtained for the addition of Citrus extract in water compared to that of the data for control meat and meat with Citrus added extract could be a further indication of the impact of interfering components in the meat reducing the accuracy of the assay.

The cooked meat without Citrus extract (control) showed a level of 0.36 ± 0.05 mg GAE g⁻¹ of meat at day 0, which remained stable up to day 1 and day 2 (Figure 1b). The meat with added Citrus extract showed significantly higher TP values during each sampling time ($p < 0.05$). Again, the difference between the values obtained in control meat and the meat added with Citrus extract was lower than the value obtained in spiked water. The values found in the unspiked meat (control) showed that the cooking process resulted in a decrease of 74% in TP values compared to the raw meat. Previous studies have shown that cooking processes can cause a decrease of phenolic compounds in vegetables (Yen, & Hung, 2000) and in total antioxidant capacity of foodstuffs (Serpen, Gökmen, & Fogliano, 2012). However, the decrease in TP values in cooked meat observed in this study is more likely due to the well-established denaturation of soluble proteins that occurs during cooking (Tornberg, 2005) leading to a lower response to the FC reagent in cooked meat. The results of the experiments showed higher TP values for the meat with Citrus extract added both in raw and particularly in cooked meat. Therefore if substantial migration of phenolic compounds from the tray surface into meat occurs it should be possible to detect an increase in phenolic content of the meat.

The experiment carried out to quantify the release of phenolic compounds into cooked meat in contact with the trays stored at 4 °C for 2 days (see section 2.4), showed TP values at day 2 of 0.345 ± 0.058 mg GAE g⁻¹ for meat stored on PET trays, 0.379 ± 0.077 mg GAE g⁻¹ for meat stored on PET-CIT trays, 0.349 ± 0.075 mg GAE g⁻¹ for meat stored on PET-PA trays and 0.404 ± 0.061 mg GAE g⁻¹ for meat stored on PET-PA-CIT trays. Although the TP values

of the meat in contact with the PET-CIT and PET-PA-CIT trays were always numerically higher than those of the meat from the PET and PET-PA trays, the differences were not statistically significant. These results suggest that while a release of phenolic substances from the trays could be detected by FC assay, the high degree of variability due to the presence of interfering components in meat meant that the differences between meat placed on uncoated and coated trays were not statistically significant.

3.2 Kinetics of release of total phenol from the trays into water

The release of TP from PET-CIT and PET-PA-CIT trays into water occurred rapidly during the first 20 min of contact, with the release from PET-PA-CIT trays being significantly higher ($p < 0.01$) than that from PET-CIT trays (Figure 2). After 20 min, the release from the trays remained steady but the release from PET-PA-CIT trays was significantly lower ($p < 0.01$) than that from PET-CIT trays from that point onwards. A previous study in our laboratory showed that a similar plasma pre-treatment resulted in a greater coating of Citrus extract on the surface of trays, with 0.46 mg cm^{-2} and 0.78 mg cm^{-2} on PET-CIT and PET-PA-CIT trays, respectively (Contini, 2013). The lower release from PET-PA-CIT trays after 20 min, despite the higher initial coating density, suggests a higher adhesion of the phenolic components of the Citrus extract to the plasma activated PET surface. On the other hand, the faster initial release may indicate that some phenolic molecules at the outer extremity of the thicker coating layer adhered less well to the surface of the plasma activated PET.

3.3 Identification and quantification of Citrus extract components

The analysis of the Citrus extract by HPLC confirmed the presence of citric acid, naringin, neohesperidin and traces of hesperidin and rhoifolin. Poncirin, ascorbic and malic

acid, if present, were not detected and no additional peaks were detected in the organic acid or flavanones elution range. However, the presence of an unknown component with high intensity required analysis by LC-MS for identification. On the basis of its deprotonated molecular mass (m/z 137), the unknown component was identified as salicylic acid; a common constituent of plants extracts (Harbourne et al., 2009). The quantification of the components by HPLC analysis revealed the presence of 25 mg of citric acid, 36 mg of salicylic acid, 1.5 mg of naringin and 1.4 mg of neohesperidin per 100 mg of Citrus extract.

3.4 Release of Citrus extract components from PET-CIT trays into meat

An attempt was made to confirm the presence and quantify by HPLC the levels of specific constituents of the Citrus extract in meat which had been placed on the PET-CIT trays for up to 2 days at 4 °C. The results of the analysis showed that the citric acid was already detectable in the meat at day 0 and its content further increased during the following days, to reach a maximum value of $370 \pm 12.5 \mu\text{g g}^{-1}$ of meat at day 2, with significantly higher values ($p < 0.01$) at day 1 and 2 compared to day 0 (Figure 3a). Salicylic acid showed an immediate release (day 0), with no significant further increase during the study; the highest value of $144 \pm 15.7 \mu\text{g g}^{-1}$ of meat was obtained at day 1. Uptake of flavanones was lower than that of the organic acids as expected considering their lower level of occurrence, with highest values of $15.1 \pm 5.4 \mu\text{g g}^{-1}$ meat for naringin and $11.1 \pm 1.5 \mu\text{g g}^{-1}$ meat for neohesperidin after 2 days of storage (Figure 3b). The results did not show any significant difference between the different storage times for flavanone uptake.

In the final experiment, the amount of Citrus extract constituents taken up by a $30 \times 30 \times 5$ mm (3g) slice of cooked turkey meat was calculated from the differences in the amounts of coating constituents extractable into water from a control PET-CIT tray and a PET-CIT tray which had been in contact with the slices of meat for 2 days at 4 °C (section

2.8). The results showed a broadly similar release between citric and salicylic acid after 2 days (Figure 4a). The highest values of release, expressed on the basis of 3g meat, were $453 \pm 67.0 \mu\text{g g}^{-1}$ meat for citric acid, $408 \pm 29.4 \mu\text{g g}^{-1}$ meat for salicylic acid. The flavanones showed similar trends, with a release of $21.2 \pm 3.7 \mu\text{g g}^{-1}$ meat for naringin and $18.1 \pm 3.3 \mu\text{g g}^{-1}$ meat for neohesperidin (Figure 4b). The values calculated for citric acid, salicylic acid, naringin and neohesperidin, measured directly in the cooked meat (Figure 3) represented 82%, 35%, 71% and 61%, respectively, of these values calculated indirectly through the release into water (Figure 4). The amount of the substances released into meat, calculated as the difference between the release in water from a tray after contact with meat and a tray that has not been in contact with meat, could have been overestimated due to the presence of a deposit remaining on the surface of the tray after its contact with the meat, which may have hampered the release of the residual Citrus extract constituents. This hypothesis could explain the apparent inconsistency of the results of the two experiments.

The total amount of Citrus extract on the PET trays has been previously calculated gravimetrically as 0.46 mg cm^{-2} (Contini, 2013). Using this value and the percentage of each constituent of the Citrus extract calculated from HPLC analysis in the present study, in the case of a complete release of the components, their amount in meat was estimated as $345 \mu\text{g g}^{-1}$ of meat for citric acid, $497 \mu\text{g g}^{-1}$ of meat for salicylic acid, $20 \mu\text{g g}^{-1}$ of meat for naringin and $19 \mu\text{g g}^{-1}$ of meat for neohesperidin. A comparison between the amount measured directly in the meat and the values estimated on the basis of the Citrus extract coating density indicated an almost complete release for citric acid, while the releases of naringin, neohesperidin and salicylic acid were 75%, 58% and 30% respectively of their estimated levels in the coating. The results indicate that the antioxidant effect of the packaging may well be based on the release of the active substances from the trays into the food. The high polarity of citric acid and flavanones, due to their carboxyl and glycoside groups,

respectively, may explain their rapid transfer into the water phase of cooked meat. Moreover, the results of the experiment regarding the kinetics of TP release into water (see section 3.2) also indicated the propensity of phenolic components of Citrus extract to migrate rapidly into the aqueous phase. The lower release of salicylic acid could be explained by the lower polarity of this molecule (Shalmashi, & Eliassi, 2008).

While the released substances can exert their antioxidant activity on the surface of the meat, the antioxidant activity of the packaging could be also due to their diffusion deep into the tissue of the meat. Regarding the antioxidant activity of the individual components, citric acid has been described as an inhibitor of lipid oxidation in fatty food (Hras, Hadolin, Knez, & Bauman, 2000) and studies on the antioxidant mechanism of citric acid have revealed that its carboxyl or hydroxyl groups can exert a binding effect on metals thus inhibiting their catalysis of oxidative reactions (Vareltzis, Hultin, & Autio, 2008). Flavanones have antioxidant properties and their mechanism of action as free radical scavengers is well documented (Cao, Sofic, & Prior, 1997). Salicylic acid has also been shown to have an antioxidant activity in biological systems mainly by the stimulation of enzymes, such as superoxide dismutase, catalase and peroxidase, as part of the antioxidative defense mechanism (Tareen, Abbasi, & Hafiz, 2012). Previous work in our laboratory has established that the Citrus extract coated PET trays can substantially reduce lipid oxidation in cooked turkey meat over 4 days of storage period and its antioxidant effect is evident from the earliest stages of the storage (Contini et al., 2012). The rapid release of the antioxidant species from the coating into the meat, observed in the present work is consistent with and indeed seems a pre-requisite for the immediacy of the antioxidant effect observed in the earlier studies. However a further confirmation of the mechanism of the antioxidant effect of the packaging came from the experiment on the kinetic of release of Citrus extract phenolics into water, which showed a lower release from PET-PA-CIT trays compared to PET-CIT

trays (Figure 2). In fact, previous experiments showed that plasma pre-treatment enhanced the antioxidant effect of the active packaging (Contini, 2013). These results suggested that an antioxidant effect may also be exerted by the Citrus extract components which remained on the tray surface, where they could reduce lipid oxidation on the surface of the meat in contact with the packaging, and that the extent of this effect depended on the amount of the coated substances (0.46 mg cm^{-2} and 0.78 mg cm^{-2} on PET-CIT and PET-PA-CIT trays, respectively).

CONCLUSION

The major components in the commercial Citrus extract, Citrox, quantified by HPLC, are citric acid, salicylic acid, naringin and neohesperidin. The mechanism of action of antioxidant active packaging containing Citrus extract appears to involve release to the antioxidant molecules from the packaging into the food from its earliest exposure to the packaging. At the same time, the lower release into water of Citrus extract components from plasma pre-treated trays combined with their higher antioxidant effect suggests a contribution to antioxidant activity from the substances remaining on the PET surface.

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References

- *Camo, J., Beltrán, J. A., & Roncalés, P. (2008). Extension of the display life of lamb with an antioxidant active packaging. *Meat Science*, 80, 1086-1091.
- Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behaviour of flavonoids: structure-activity relationships. *Free Radical Biology & Medicine*, 22, (5), 749-760.
- Chen, X., Lee, D. S., Zhu, X., & Yam, K. L. (2012). Release kinetics of tocopherol and quercetin from binary antioxidant controlled-release packaging films. *Journal of Agricultural and Food Chemistry*, 60, 3492-3497.
- Contini, C. (2013). Characterisation of Citrus Extract-based Active Packaging to reduce Lipid Oxidation in Cooked Meats. PhD Thesis, School of Agriculture and Food Science, University College Dublin, Ireland.
- *Contini, C., Katsikogianni, M. G., O'Neill, F. T., O'Sullivan, M., Dowling, D. P., & Monahan, F. J. (2012). PET trays coated with Citrus extract exhibit antioxidant activity with cooked turkey meat. *Food Science and Technology*, 47, 471-477.
- De Kruijf, N., Van Beesty, M., Rijky, R., Sipiläinen-Malm, T., Paseiro Losada, P., & De Meulenaer, B. (2002). Active and intelligent packaging: applications and regulatory aspects. *Food Additives and Contaminants*, 19, 144-162.
- Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method for the isolation and purification of total oil from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.
- Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas, G., & Georgakis, S. A. (2007). Effect of rosemary extract, chitosan and α -tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat Science*, 76, 172-181.

418 Gray, J. I., & Pearson, A. M. (1987) Rancidity and warmed-over flavor. In: A. M. Pearson, &
 419 T. R. Dutson (Eds.), *Advances in Meat Research*, vol. 3 (pp. 221–270). New York: Van
 420 Nostrand Reinhold Company.

421 *Harbourne, N., Marete, E., Jacquier, J. C., & O’Riordan, D. (2009). Effect of drying on the
 422 phenolic constituent of meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*). *Food*
 423 *Science and Technology*, 42, 1468-1473.

424 Hras, A. R., Hadolin, M., Knez, Z., & Bauman, D. (2000). Comparison of antioxidative and
 425 synergistic effects of rosemary extract with α -tocopherol, ascorbyl palmitate and citric acid in
 426 sunflower oil. *Food Chemistry*, 71, 229-233.

427 Jang, A., Liu, X. D., Shin, M. H., Lee, B. D., Lee, S. K., Lee, J. H., & Jo, C. (2008).
 428 Antioxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb
 429 extract mix. *Poultry Science*, 87, (11), 2382-2389.

430 Kerry, J. P., O’Grady, M. N., & Hogan, S. A. (2006). Past, current and potential utilisation of
 431 active and intelligent packaging systems for meat and muscle-based products: A review.
 432 *Meat Science*, 74, 113-130.

433 Koontz, J. L., Moffitt, R. D., Marcy, J. E., O’Keefe, S. F., Duncan S. E., & Long T. E.
 434 (2010). Controlled release of α -tocopherol, quercetin, and their cyclodextrin inclusion
 435 complexes from linear low-density polyethylene (LLDPE) films into a coconut oil model
 436 food system. *Food Additives and Contaminants*, 27, (11), 1598-1607.

437 Lee, D. S. (2005). Packaging containing natural antimicrobial or antioxidant agents. In Han J.
 438 (ed.) *Innovation in Food Packaging* (pp. 108-119). Elsevier Science and Technology Books.

439 Lowry, O. H., Rosebrough, N. J., Lewis Farr, A., & Randall, R. J. (1951). Proteine
 440 measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, 193, (1),
 441 265-275.

442 McCance, & Widdowson (2002). The Composition of Foods, 6th Summary Edition.
 443 Cambridge: Royal Society of Chemistry.

444 *Mexis, S. F., Chouliara, E., & Kontominas, M. G. (2012). Shelf life extension of ground
 445 chicken meat using an oxygen absorber and a citrus extract. *Food Science and Technology*,
 446 49, 21-27.

447 *Nerín, C., Tovar, L., & Salafranca, J. (2008). Behaviour of a new antioxidant active film
 448 versus oxidizable model compounds. *Journal of Food Engineering*, 84, 313-320.

449 Nijveldt, R. J., van Nood, E., van Hoorn, D. E. C., Boelens, P. G., van Norren, K., & van
 450 Leeuwen, P. A. M. (2001). Flavonoids: a review of probable mechanisms of action and
 451 potential applications. *American Journal Clinical and Nutrition*, 74, 418-25.

452 Okabe, Y., Watanabe, A., Shingu, H., Kushibiki, S., Hodate, K., Ishida, M., Ikeda, S., &
 453 Takeda, T. (2002). Effects of α -tocopherol level in raw venison on lipid oxidation and
 454 volatiles during storage. *Meat Science*, 62, 457-462.

455 Pettersen, M. K., Mielnik, M. B., Eie, T., Skrede, G., & Nilsson, A. (2004). Lipid Oxidation
 456 in frozen, mechanically deboned turkey meat as affected by packaging parameters and
 457 storage conditions. *Poultry Science*, 83, 1240-1248.

458 Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of
 459 antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural*
 460 *and Food Chemistry*, 53, (10), 4290-4302.

461 Ruban, S. W. (2009). Lipid peroxidation in muscle food - an Overview. *Global Veterinaria*,
 462 3, (6), 509-513.

463 Shalmashi, A., & Eliassi, A. (2008). Solubility of salicylic acid in water, ethanol, carbon
 464 tetrachloride, ethyl acetate, and xylene. *Journal of Chemical & Engineering Data*, 53, 199-
 465 200.

466

467 Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors associated with
 468 fresh meat color: a Review. *Journal of Food Quality*, 6, 211-237.

469 Serpen, A., Gökmen, V., & Fogliano, V. (2012). Total antioxidant capacities of raw and
 470 cooked meats. *Meat Science*, 90, 60-65.

471 Soto-Cantú, C. D., Graciano-Verdugo, A. Z., Peralta, E., Islas-Rubio, A. R., González-
 472 Córdova, A., González-León, A., & Soto-Valdez H. (2008). Release of butylated
 473 hydroxytoluene from an active film packaging to asadero cheese and its effect on oxidation
 474 and odor stability. *Journal of Dairy Science*, 91, 11-19.

475 Szydłowska-Czerniak, A., Tułodziecka, A., & Szłyk, E. (2012). Determination of antioxidant
 476 capacity of unprocessed and processed food products by spectrophotometric methods. *Food*
 477 *Analysis and Methods*, 5, 807-813.

478 Tareen, M. J., Abbasi, N. A., & Hafiz, I. A. (2012). Postharvest application of salicylic acid
 479 enhanced antioxidant enzyme activity and maintained quality of peach cv. 'Flordaking' fruit
 480 during storage. *Scientia Horticulturae*, 142, 221-228.

481 Tornberg, E. (2005). Effect of heat of meat proteins – Implications on structure and quality of
 482 meat products. *Meat Science*, 70 (3), 493-508.

483 Vareltzis, P., Hultin, H. O., & Autio, W. R. (2008). Hemoglobin-mediated lipid oxidation of
 484 protein isolates obtained from cod and haddock white muscle as affected by citric acid,
 485 calcium chloride and pH. *Food Chemistry*, 108, 64-74.

486 Yen, G. C., & Hung, C. Y. (2000). Effects of alkaline and heat treatment on antioxidative
 487 activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.). *Food*
 488 *Research International*, 33, 487-492.

489 Van Aardt, M., Duncan, S. E., Marcy, J. E., Long, T. E., O'Keefe, S. F., & Sims, S. R.
 490 (2007). Release of antioxidants from poly(lactide-co-glycolide) films into dry milk products

491 and food simulating liquids. *International Journal of Food Science and Technology*, 42, (11),
492 1327-1337.
493

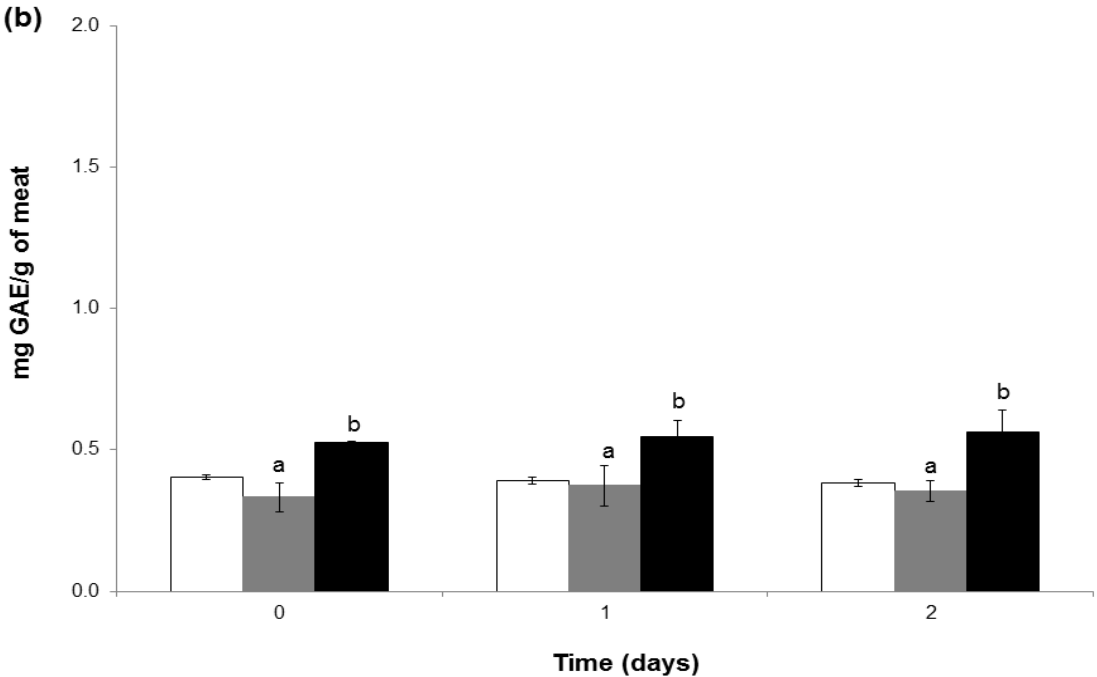
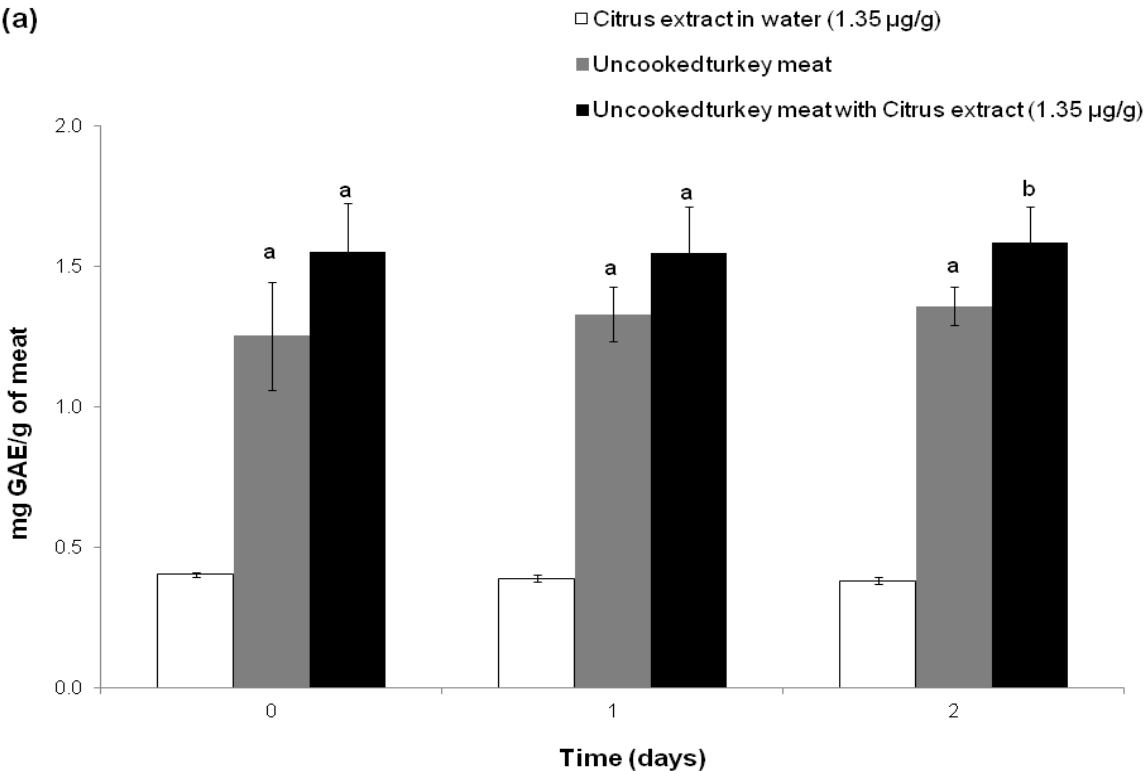
Figure 1. Effect of the direct addition of Citrus extract on the total phenol content of (a) raw and (b) cooked turkey meat, stored for 2 days at 4 °C. White column, Citrus extract added into water (control); grey column, turkey meat; black column, turkey meat with Citrus extract. Bars indicate mean \pm SD. ^{a,b} Within each storage time, bars with different letters are significantly different due to treatment. Within each treatment there was no significant difference due to storage time.

Figure 2. Release of the total phenols from the PET-CIT and PET-PA-CIT trays into water, stored for 2 days at 4 °C. Points indicate mean \pm SD. ^{a,b} Within each storage time, bars with different letters are significantly different due to treatment. ^{r,s,t,u,v,w,x,y,z} Within each treatment, bars with different letters are significantly different due to storage time.

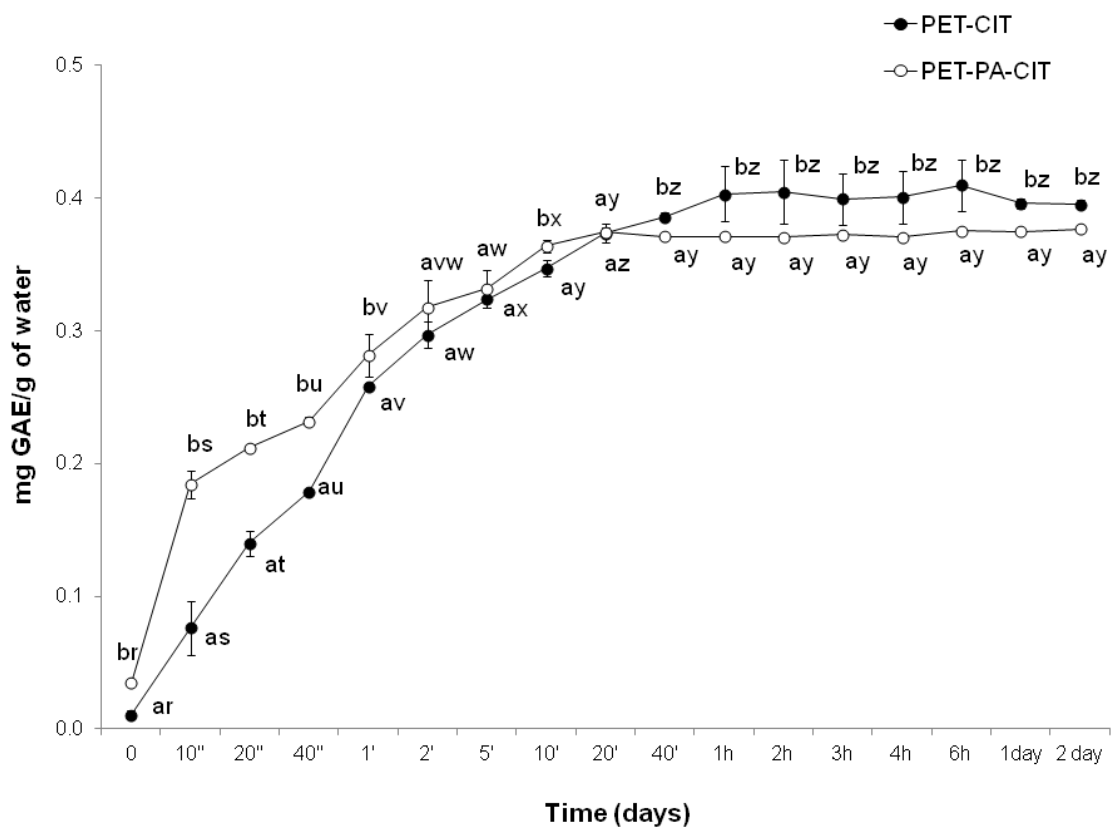
Figure 3. Release of (a) organic acids and (b) flavanones from PET-CIT trays into meat stored for up to 2 days at 4 °C. Bars indicate mean \pm SD. ^{y,z} Within each treatment, bars with different letters are significantly different due to the storage time.

Figure 4. Release of (a) organic acids and (b) flavanones from PET-CIT trays into meat stored for 2 days at 4 °C, calculated from the release into water. Bars indicate mean \pm SD. Within each treatment there was no difference due to storage time.

Figure 1. Contini et al.



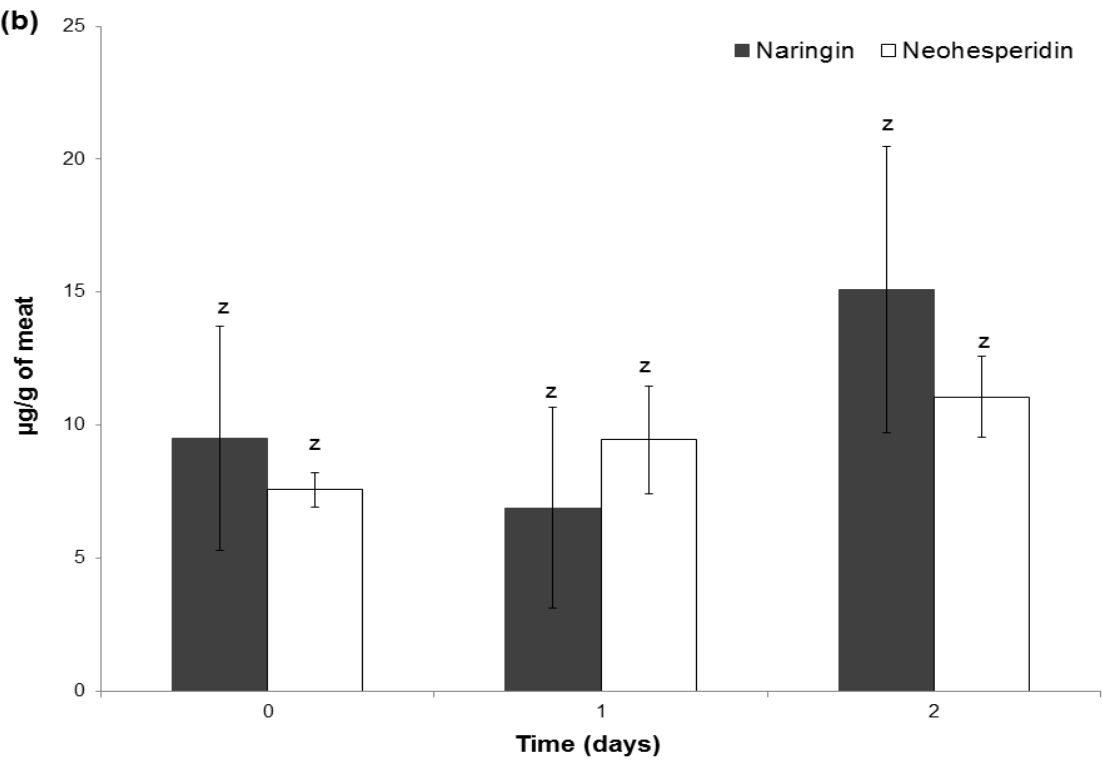
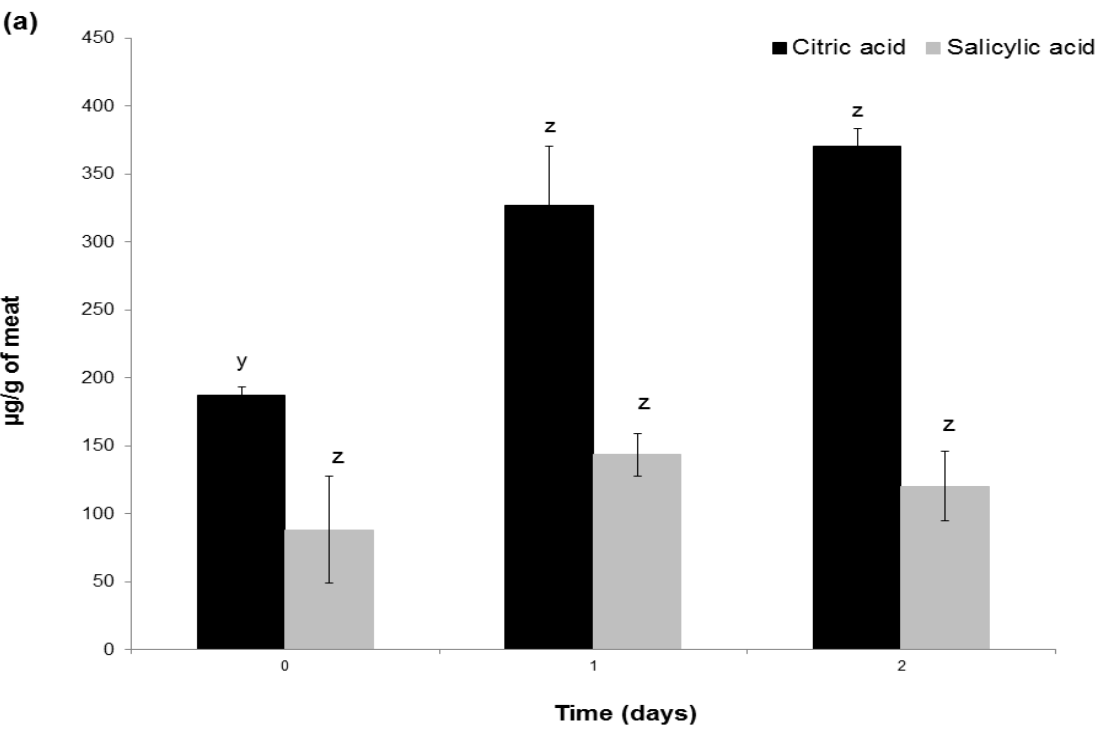
519 **Figure 2.** Contini et al.

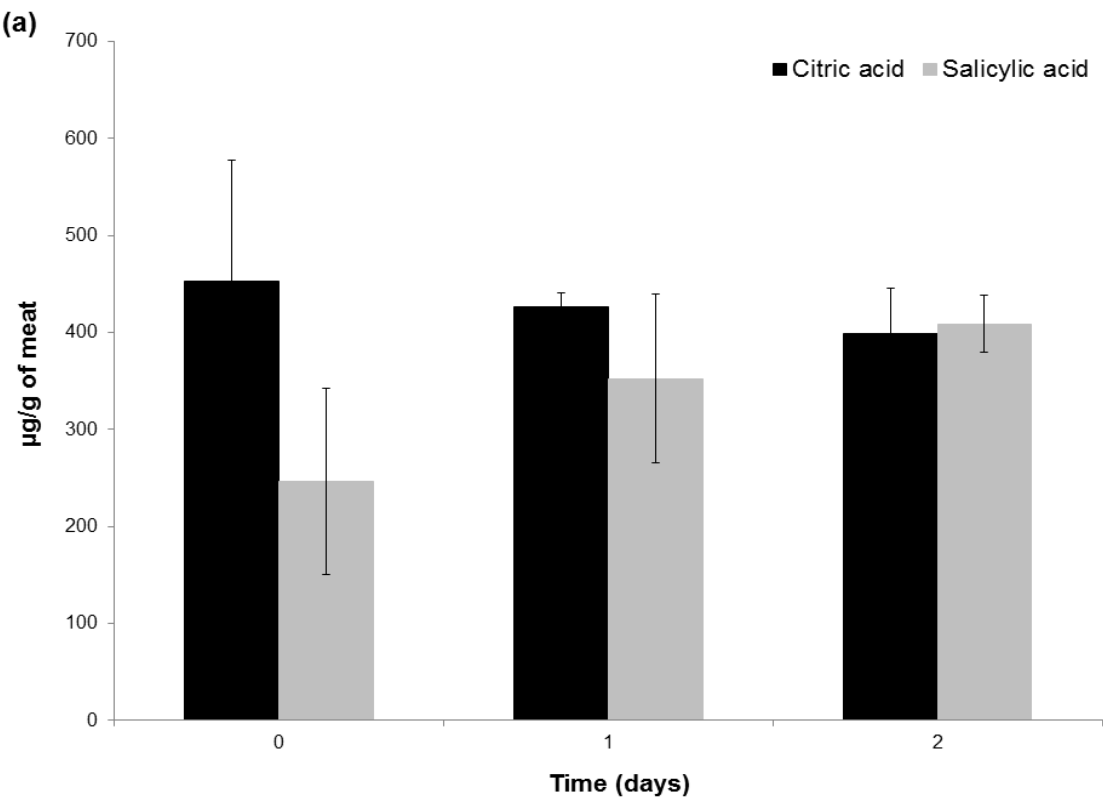


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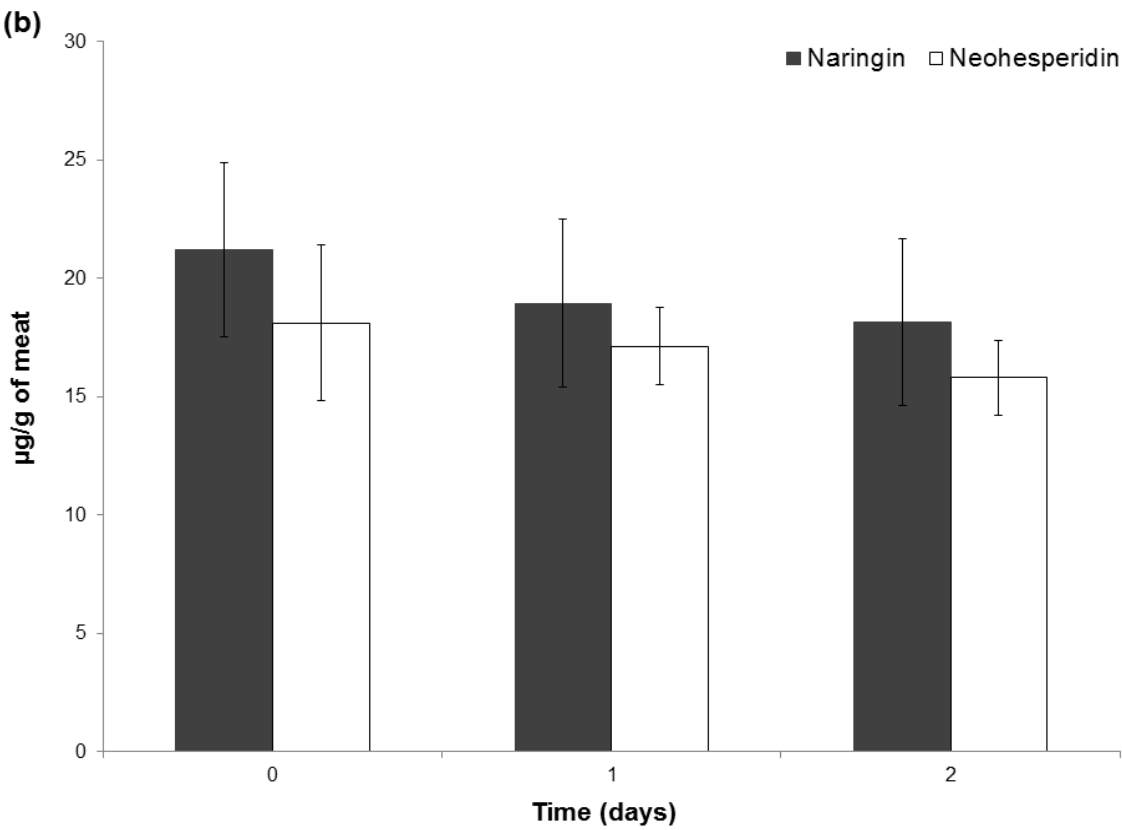
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522 **Figure 3.** Contini et al.





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